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2. Patent application number *(The Patent Office will fill in this part)*	**9818732.1** 27 AUG 1998
3. Full name, address and postcode of the or of each applicant *(underline all surnames)*	THE UNIVERSITY OF PORTSMOUTH HIGHER EDUCATION CORPORATION UNIVERSITY HOUSE WINSTON CHURCHILL AVENUE PORTSMOUTH PO1 2UP
Patents ADP number *(if you know it)*	7582115001
If the applicant is a corporate body, give the country/state of its incorporation	ENGLAND
4. Title of the invention	COLLECTIONS OF COMPOUNDS
5. Name of your agent *(if you have one)*	MEWBURN ELLIS
"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	YORK HOUSE 23 KINGSWAY LONDON WC2B 6HP
Patents ADP number *(if you know it)*	109006

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Claim*(s)*	5
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Priority documents	0
Translations of priority documents	0
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	1
Request for preliminary examination and search *(Patents Form 9/77)*	0
Request for substantive examination *(Patents Form 10/77)*	0
Any other documents *(Please specify)*	0

11. I/We request the grant of a patent on the basis of this application.

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Date 27 August 1998

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COLLECTIONS OF COMPOUNDS

This invention relates to pyrrolobenzodiazepines, to methods of synthesizing these compounds on solid supports, and to collections of these compounds. This invention further relates to methods for identifying and isolating pyrrolobenzodiazepine compounds with useful and diverse activities from such collections.

Background to the invention

Compounds having biological activity can be identified by screening diverse collections of compounds (i.e. libraries of compounds) produced through synthetic chemical techniques. Such screening methods include methods wherein the library comprises a plurality of compounds synthesized at specific locations on the surface of a solid support whereby a receptor is appropriately labelled to bind to and identify a compound, e.g., fluorescent or radioactive labels. Correlation of the labelled receptor bound to the support and its location on the support identifies the binding compound (US 5,143,854).

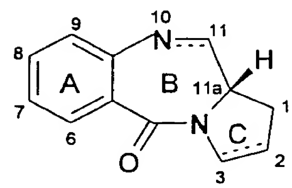
Central to these methods is the screening of a multiplicity of compounds in the library and the ability to identify the structures of the compounds which have a requisite biological activity. In order to facilitate synthesis and identification, the compounds in the library are typically formed on solid supports. Usually each such compound is covalently attached to the support via a cleavable or non-cleavable linking arm. The libraries of compounds can be

screened either on the solid support or as cleaved products to identify compounds having good biological activity.

A particular class of compounds which would be useful for inclusion in screening libraries are pyrrolobenzodiazepines (PBDs). PBDs have the ability to recognise and bond to specific sequences of DNA; the most preferred sequence is PuGPu (Purine-Guanine-Purine). The first PBD antitumour antibiotic, anthramycin, was discovered in 1965 (Leimgruber *et al.*, 1965 *J. Am. Chem. Soc.*, **87**, 5793-5795; Leimgruber *et al.*, 1965 *J. Am. Chem. Soc.*, **87**, 5791-5793). Since then, a number of naturally occurring PBDs have been reported, and over 10 synthetic routes have been developed to a variety of analogues (Thurston *et al.*, 1994 *Chem. Rev.* **1994**, 433-465). Family members include abbeymycin (Hochlowski *et al.*, 1987 *J. Antibiotics*, **40**, 145-148), chicamycin (Konishi *et al.*, 1984 *J. Antibiotics,* **37**, 200-206), DC-81 (Japanese Patent 58-180 487; Thurston *et al.*, 1990, *Chem. Brit.*, **26**, 767-772; Bose *et al.*, 1992 *Tetrahedron*, **48**, 751-758), mazethramycin (Kuminoto *et al.*, 1980 *J. Antibiotics*, **33**, 665-667), neothramycins A and B (Takeuchi *et al.*, 1976 *J. Antibiotics*, **29**, 93-96), porothramycin (Tsunakawa *et al.*, 1988 *J. Antibiotics*, **41**, 1366-1373), prothracarcin (Shimizu *et al*, 1982 *J. Antibiotics*, **29**, 2492-2503; Langley and Thurston, 1987 *J. Org. Chem.*, **52**, 91-97), sibanomicin (DC-102)(Hara *et al.*, 1988 *J. Antibiotics*, **41**, 702-704; Itoh *et al.*, 1988 *J. Antibiotics*, **41**, 1281-1284), sibiromycin (Leber *et al.*, 1988 *J. Am. Chem. Soc.*, **110**, 2992-

2993) and tomamycin (Arima *et al.*, 1972 *J. Antibiotics*, **25**, 437-444).

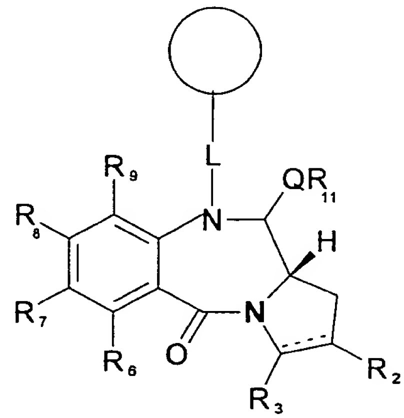
PBDs are of the general structure:



They differ in the number, type and position of substituents, in both their aromatic A rings and pyrrolo C rings, and in the degree of saturation of the C ring. There is either an imine (N=C), a carbinolamine (NH-CH(OH)) or a carbinolamine methyl ether (NH-CH(OMe))at the N10-C11 position which is the electrophilic centre responsible for alkylating DNA. All of the known natural products have an (*S*)-configuration at the chiral C11a position which provides them with a right-handed twist when viewed from the C ring towards the A ring. This gives them the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, 1975 in *Antibiotics III*. Springer-Verlag, New York, pp. 3-11 ; Hurley and Needham-VanDevanter, 1986 *Acc. Chem. Res.*, **19**, 230-237). Their ability to form an adduct in the minor groove, enables them to interfere with DNA processing, hence their use as antitumour agents.

Disclosure of the Invention

A first aspect of the present invention relates to compounds of formula (I):



(I)

wherein:

R_2 and R_3 are independently selected from: H, R, OH, OR, =O, =CH-R, $=CH_2$, CH_2-CO_2R, CH_2-CO_2H, CH_2-SO_2R, $O-SO_2R$, CO_2R, COR and CN, and there is optionally a double bond between C2 and C3;

R_6, R_7, R_8 and R_9 are independently selected from H, R, OH, OR, halo, nitro, amino, Me_3Sn;

R_{11} is either H or R;

Q is S, O or NH;

L is a linking group, or less preferably a single bond;

○ is a solid support;

where R is a lower alkyl group having 1 to 10 carbon atoms, or an alkaryl group (i.e. an alkyl group with one or more aryl substituents) preferably of up to 12 carbon atoms, whereof the alkyl group optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system, or an aryl group, preferably of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally contains one or more

hetero atoms, which may form part of, or be, a functional group; and

where one or more of R_2, R_3, R_6, R_7 and R_8 may alternatively be independently X-Y-A-, where X is selected from -COOH, NHZ, SH, or OH, where Z is either H or an amine protecting group, Y is a divalent alkylene or aryl group such that HY = R, and A is O, S, NH, or a single bond.

If R is an aryl group and contains a hetero atom, then R is a heterocyclic group. If R is an alkyl chain, and contains a hetero atom, the hetero atom may be located anywhere in the alkyl chain, e.g. $-O-C_2H_5$, $-CH_2-S-CH_3$, or may form part of, or be, a functional group, e.g. carbonyl, hydroxy, cyano, ester.

R and HY groups are preferably independently selected from a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group, preferably of up to 12 carbon atoms, or an aryl group, preferably of up to 12 carbon atoms, optionally substituted by one or more halo, hydroxy, amino, or nitro groups. It is more preferred that R and HY groups are independently selected from a lower alkyl group having 1 to 10 carbon atoms optionally substituted by one or more halo, hydroxy, amino, or nitro groups. It is particularly preferred that R or HY are unsubstituted straight or branched chain alkyl groups, having 1 to 10, preferably 1 to 6, and more preferably 1 to 4, carbon atoms, e.g. methyl, ethyl, propyl, butyl.

Alternatively, R_6, R_7, R_8 and R_9 may preferably be independently selected from R groups with the following

structural characteristics:

(i) an optionally substituted phenyl group;

(ii) an optionally substituted ethenyl group;

(iii) an ethenyl group conjugated to an electron sink.

The term 'electron sink' means a moiety covalently attached to a compound which is capable of reducing electron density in other parts of the compound. Examples of electron sinks include cyano, carbonyl and ester groups.

The term 'amine protecting group' has the meaning usual in synthetic chemistry, particularly synthetic peptide chemistry. It means any group which may be covalently bound to the nitrogen atom of the amine grouping, and permits reactions to be carried out upon the molecule containing this grouping without its removal. Nevertheless, it is able to be removed from the nitrogen atom without affecting the remainder of the molecule. Suitable nitrogen protecting groups for the present invention include Fmoc (9-fluorenylmethoxycarbonyl), Nvoc (6-nitroveratryloxycarbonyl), Teoc (2-trimethylsilylethyloxycarbonyl), Troc (2,2,2-trichloroethyloxycarbonyl), Boc (t-butyloxycarbonyl), CBZ (benzyloxycarbonyl), Alloc (allyloxycarbonyl) and Psec (2(-phenylsulphonyl)ethyloxycarbonyl). Other suitable groups are described in Protective Groups in Organic Synthesis, T Green and P Wuts, published by Wiley, 1991 which is incorporated herein by reference.

It is preferred that in compounds of formula I, if one of R_2,

R_3, R_6, R_7 and R_8 is to be X-Y-A-, then it is either R_2 or R_8 that is X-Y-A-.

In compounds of formula **I**, Q is preferably O, and R_{11} is preferably H. Independently, R_6 and R_9 are preferably H, and R_7 is preferably an alkoxy group, and more preferably methoxy or ethoxy. It is further preferred that R_2 and R_3 are H.

A second aspect of the invention relates to compounds of formula **I** as defined in the first aspect of the invention except that one or more of R_2, R_3, R_6, R_7 and R_8 are independently:

$$H-(T)_n-X'-Y-A-$$

where:

Y and A are as defined in the first aspect of the invention;

X' is CO, NH, S or O,;

T is a combinatorial unit;

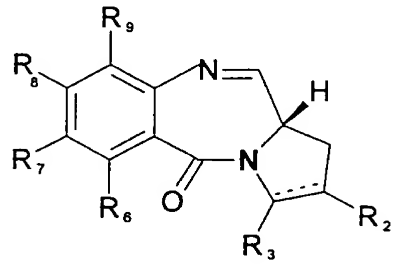
and n is a positive integer.

In compounds of formula **I** according to the second aspect, it is preferred that R_2 and/or R_8 are independently:

$$H-(T)_n-X'-Y-A-$$

It is preferred that X' is either CO or NH. n may preferably be from 1 to 16, and more preferably from 3 to 14.

A third aspect of the present invention relates to compounds of formula **II**:



(II)

preferably made from a compound of formula I as described in the first or second aspect of the invention by removing the compound of formula **II** from the solid support by cleaving the linking group L, where R_2, R_3, R_6, R_7, R_8, and R_9 are as defined in the first or second aspect of the invention.

A fourth aspect of the present invention is a method of making a compound according to the third aspect of the invention from a compound of formula **I** as described in the first or second aspect of the invention by removing the compound of formula **II** from the solid support by cleaving the linking group L.

A fifth aspect of the invention relates to a compound of formula **II** as described in the third aspect of the invention for use in a method of therapy. Conditions which may be treated include gene-based diseases, including neoplastic diseases and, for example Alzheimer's disease, and bacterial, parasitic and viral infections.

In accordance with this aspect of the present invention, the compounds provided may be administered to individuals. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one

symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosages etc., is within the responsibility of general practitioners and other medical doctors.

A compound may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to the active ingredient, i.e. a compound of formula **II**, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous or intravenous.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solutions, or glycols such as ethylene

glycol, propylene glycol or polyethylene glycol may be included. Capsules may include a solid carrier such as gelatin.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and which has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection or Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

A sixth aspect of the present invention relates to the use of a compound of formula **II** as described in the third aspect of the present invention in the preparation of a medicament for the treatment of a gene-based disease or a bacterial, parasitic or viral infection. The preparation of a medicament is described in relation to the fourth aspect of the invention.

In further aspects, the invention provides processes for preparing compounds according to the first and second aspects of the present invention.

Solid support

The term 'solid support' refers to a material having a rigid or semi-rigid surface which contains or can be derivatized to

contain reactive functionality which can serve to covalently link a compound to the surface thereof. Such materials are well known in the art and include, by way of example, silicon dioxide supports containing reactive Si-OH groups, polyacrylamide supports, polystyrene supports, polyethyleneglycol supports, and the like. Such supports will preferably take the form of small beads, pins/crowns, laminar surfaces, pellets, disks. Other conventional forms may be used.

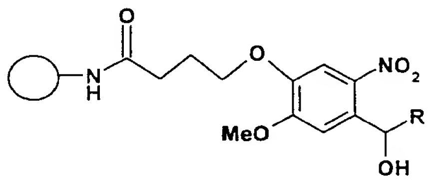
Linker group

The linking groups preferred for the present application are ones which contain at least one covalent bond which can be readily broken by specific chemical reactions, or other changes (e.g. light or a pH change), thereby providing for liberation of compounds free from the solid support. The chemical reactions employed to break the covalent bond are selected so as to be specific for the desired bond breakage thereby preventing unintended reactions occurring elsewhere in the molecule. The linking group is selected relative to the synthesis of the compounds formed on the solid support so as to prevent premature cleavage of the compound from the solid support as well as to avoid interference with any of the procedures employed during synthesis of the compound on the support.

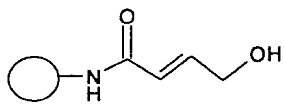
Examples of linking groups are set out below, along with suggested cleavage method(s) for the linking group. These groups are commercially available or have been reported in the

literature. After conversion to the appropriate chloroformate, for example by reaction with triphosgene in the presence of pyridine, they can be used to attach to anthranilic acids (for use in providing the protected A-rings of pyrrolobenzodiazepines) via carbamate linkages.

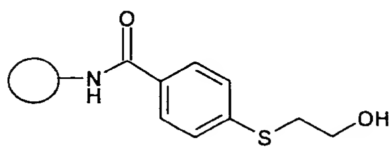
Cleavage Conditions



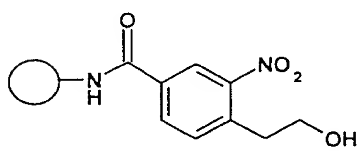
hv photolysis [1]



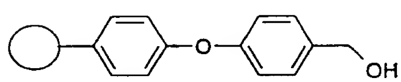
Pd(0), Nuc. [2]



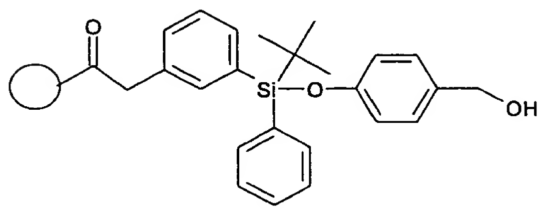
mCPBA / base [3]



base [4]



DDQ or dil. TFA [5]



TBAF [6]

References:

1. Holmes, C.P., Jones, D.G., "Reagents for Combinatorial Organic Synthesis: Development of a New O-Nitrobenzyl Photolabile Linker for Solid Phase Synthesis", *J. Org. Chem.*,

60, 2318-2319 (1995).

2. Hauske, J.R., Dorff, P.A., "Solid Phase CBZ Chloride Equivalent. A New Matrix Specific Linker", *Tetrahedron Letters*, 36, 10, 1589-1592 (1995).

3. Kunz, H., Dombo, B., "Solid Phase Synthesis of Peptide and Glycopeptides on Polymeric Supports with Allylic Anchor Groups", *Angew Chem Int Ed Engl*, 5, 711 (1988).

4. Garcia-Echeverria, C., "A Base Labile Handle for Solid Phase Organic Chemistry", *Tetrahedron Letters*, 38, 52, 8933-8934 (1997).

5. (a) Albericio, F., Giralt, E., Eritja, R., *Tetrahedron Letters, 32, 1515 (1991).*

(b) Albericio, F., Robles, J., Fernandez-Forner, Y., Palom, C., Celma, E., Pedroso, E., Giralt, E., Eritja, R., *Peptides 1990, Proc 21st Eur. Pept. Symp.*, S134, (1991).

6. Mullen, D. G, Barany, G., "A New Fluoridolyzable Anchoring Linkage for Orthogonal Solid-Phase Peptide Synthesis: Design, Preparation, and Application of the N-(3 or 4)-[[4-(Hydroxymethyl)phenoxy]-tert-butylphenylsilyl]phenyl Pentanedioic Acid Monoamide (Pbs) Handle", *J. Org. Chem.*, 53, 5240 (1988).

All these documents are incorporated herein by reference.

<u>Combinatorial Unit</u>

The term 'combinatorial unit' means any monomer unit which can be used to build a chain as shown in a compound of formula **I** as defined in the second aspect of the present invention, or a compound of formula **II**, when derived from a compound of

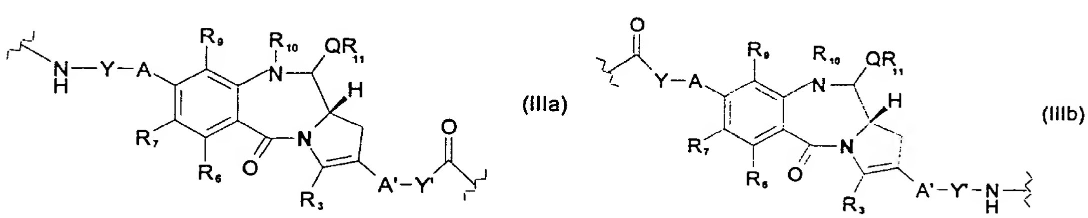
formula I as defined in the second aspect of the present invention. The chain usually attached by the PBD core by a joining group. Examples of molecules suitable for such chain building is found in Schreiber *et al.* (*JACS*, 120. 1998, pp.23-29), which is incorporated herein by reference. An important example of a unit is an amino acid residue. Chains may be synthesised by means of amine-protected amino acids. Fmoc protected amino-acids are available from a number of sources, such as Sigma and Nova Biochem. Both natural and unnatural amino acids can be used, e.g. D- and L-amino acids and heterocyclic amino acids. In particular, heterocyclic amino acids of the type found in the construction of netropsin and distamycin are of interest because of their DNA-recognition properties.

Amine units can be used to make up peptoids: see Soth, M.J. and Nowick, J.S. 1997, Unnatural oligomer libraries, *Curr. Opin, Chem. Biol.* 1, no. 1: 120-129; Zuckermann *et al.*, 1994, Discovery of Nanomolecular Ligands for 7-Transmembrane G-Protein-Coupled Receptors from a Diverse N-(Substituted)glycine Peptoid Library, *Journal of Medicinal Chemistry* 37: 2678-85; Figliozzi, GMR *et al.*, 1996, Synthesis of N-substituted Glycine Peptoid Libraries, *Methods in Enzymology*, 267: 437-47; Simon, *R.J. et al.*, 1992, Peptoids: A Modular Approach to Drug Discovery, *Proc. Natl. Acad. Sci. USA*, 89:9367-71; which documents are incorporated herein by reference.

Other combinatorial units include PNAs: P E Nielsen, *et al.*,

Science, 1991, 254, 1497; M Egholm, *et al.*, Nature, 1993, 365, 566; M Egholm *et al.*, *JACS*, 1992, 114, 1895; S C Brown, *et al.*, *Science*, 1994, 265, 777; 5. K Saha, *et al.*, *JOC*, 1993, 58, 7827; oligoureas: K Burgess, *et al.*, 1995, Solid Phase Synthesis of Unnatural Biopolymers Containing Repeating Urea Units. *Agnew. Chem. Int. Ed. Engl* 34, no. 8:907; K Burgess, *et al.*, 1997, Solid Phase Synthesis of Oligoureas; *Journal of the American Chemical Society* 119: 1556-64; and oligocarbamates: E J Moran *et al.*, 1995, Novel Biopolymers for Drug Discovery. *Biopolymers (Peptide Science); John Wiley and Sons* 37: 213-19; Cho C Y *et al.*, 1993, An Unnatural Biopolymer. *Science* 261: 1303-5; Paikoff S F *et al.*, 1996, The Solid Phase Synthesis of N-Alkylcarbamate Oligomers. *Tetrahedron Letters* 37, no. 32: 5653-56. All these documents are incorporated herein by reference.

A type of combinatorial unit of particular relevance to the present invention is one based on the pyrrolobenzodiazepine structures; these are of general formulae **IIIa** and **IIIb**:



wherein R_3, R_6, R_7, R_9, R_{11}, Q, A and Y are as defined in the first aspect of the invention, and A′ and Y′ are independently selected from the possible groups for A and Y respectively.

The present invention relates to libraries, or collections, of compounds all of which are represented by a single one of the formulae **I** or **II**. The diversity of the compounds in a library may reflect the presence of compounds differing in the identities of one or more of R_2, R_3, R_6, R_7, R_9, R_{11} and Q and/or in the identities of the combinatorial units T (when present). The number of members in the library depends on the number of variants, and the number of possibilities for each variant. For example, if it is the combinatorial units which are varied, and there are 3 combinatorial units, with 3 possibilities for each unit the library will have 27 compounds. 4 combinatorial units and 5 possibilities for each unit gives a library of 625 compounds. If for instance there is a chain of 5 combinatorial units with 17 possibilities for each unit, the total number of members in the library would be 1.4 million. A library may therefore comprise more than 1 000, 5 000, 10 000, 100 000 or a million compounds, which may be arranged as described below.

In the case of free compounds (formula **II**) the individual compounds are preferably in discrete volumes of solvents, e.g. in tubes or wells. In the case of bound compounds (formula **I**) the individual compounds are preferably bound at discrete locations, e.g. on respective pins/crowns or beads. The library of compounds may be provided on a plate which is of a suitable size for the library, or may be on a number of plates of a standard size, e.g. 96 well plates. If the number of members of the library is large, it is preferable that each well on a plate contains a number of related compounds from

the library, e.g. from 10 to 100. One possibility for this type of grouping of compounds is where only a subset of the combinatorial units, or substituents, are known and the remainder are randomised; this arrangement is useful in iterative screening processes(see below). The library may be presented in other forms that are well-known.

A further aspect of the present invention is a method of preparing a collection, or library of compounds as discussed above. If the diversity of the library is in the combinatorial units, then the library may be synthesised by the stepwise addition of protected combinatorial units to a PBD core, each step being interposed by a deprotection step. Such a method is exemplified later. If the diversity of the library is in the substituent groups, the library may be synthesised by carrying out the same synthetic methods on a variety of starting materials or key intermediates, which already possess the necessary substituent patterns.

The present invention also relates to a method of screening the compounds of formula **II** to discover biologically active compounds. The screening can be to assess the binding interaction with nucleic acids, e.g. DNA or RNA, or proteins, or to assess the affect of the compounds against protein-protein or nucleic acid-protein interactions, e.g. transcription factor DP-1 with E2F-1, or estrogen response element (ERE) with human estrogen receptor (a 66 kd protein which functions as a hormone-activated transcription factor, the sequence of which is published in the art and is generally

available). The screening can be carried out by bringing the target macromolecules into contact with individual compounds or the arrays or libraries described above, and selecting those compounds, or wells with mixtures of compounds, which show the strongest effect.

This effect may simply be the cytotoxicity of the compounds in question against cells or the binding of the compounds to nucleic acids. In the case of protein-protein or nucleic acid-protein interactions, the effect may be the disruption of the interaction studied.

Protein-protein interactions can be measured in a number of ways, e.g. FRET (fluorescence resonance energy transfer) which involves labelling one of the proteins with a fluorescent donor moiety and the other with an acceptor which is capable of absorbing the emission from the donor; the fluorescence signal of the donor will be altered depending on the interaction between the two proteins. Another method of measuring protein-protein interactions is by enzymatic labelling, using, for example, horseradish peroxidase.

The screening process may undergo several iterations by selecting the most active compounds, or group of compounds, tested in each iteration; this is particular useful when testing arrays of wells which include mixtures of related compounds. Furthermore, if the wells contain compounds for which only a subset of the combinatorial units, or substituents, are known, but the rest are randomised,

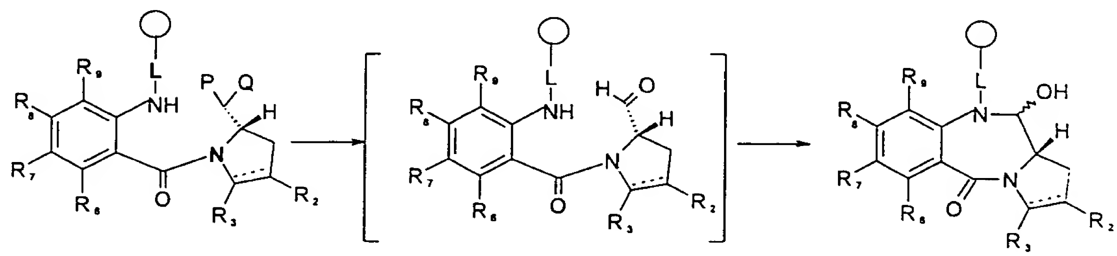
subsequent iterations can be carried out by synthesising compounds possessing the selected known (and successful) combinatorial unit, or substituent, pattern, but with further specified combinatorial units, or substituents, replacing the previously randomised combinatorial units, or substituents, adjacent the already known pattern; the remaining combinatorial units, or substituents, are randomised as in the previous iteration. This iterative method enables the identification of active members of large libraries without the need to isolate every member of the library.

A further feature of this aspect is formulation of selected compound or compounds with pharmaceutically acceptable carriers or diluents.

A further aspect of the present invention relates to the use of compounds of formula **II** in target validation. Target validation is the disruption of an identified DNA sequence to ascertain the function of the sequence, and a compound of formula **II** can be used to selectively bind an identified sequence, and thus disrupt its function.

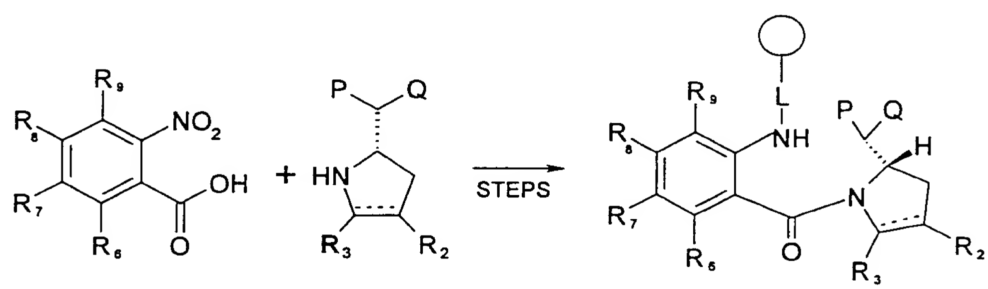
Synthesis Methods

A key step in a preferred route to compounds of formula **I** is a cyclisation to produce the B-ring, involving generation of an aldehyde (or functional equivalent thereof) at what will be the 11-position, and attack thereon by the pro-10-nitrogen:



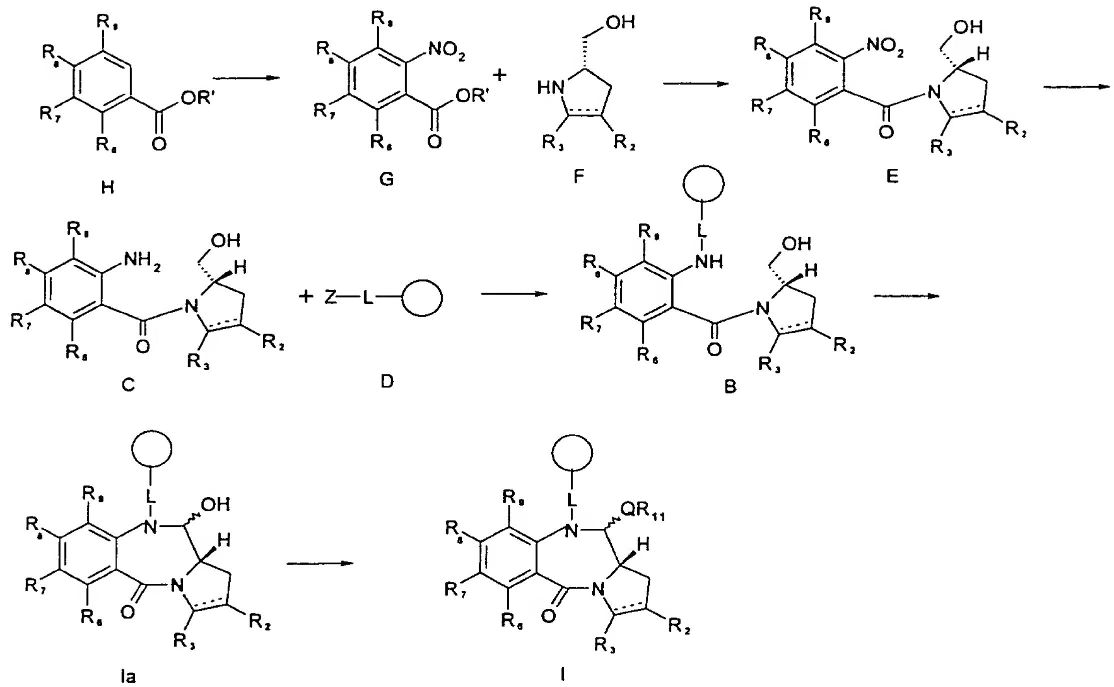
The "masked aldehyde" -CPQ may be an acetal or thioacetal, in which case the cyclisation involves unmasking. Alternatively, it may be an alcohol -CHOH, in which case the reaction involves oxidation, e.g. by means of TPAP or DMSO (Swern oxidation).

The masked aldehyde compound can be produced by condensing a corresponding 2-substituted pyrrolidine with a 2-nitrobenzoic acid:



The nitro group can then be reduced to $-NH_2$ and reacted with a suitable linking group attached to a solid support, e.g. a chloroformate, which thereby links the structure to the solid support.

A process involving the oxidation-cyclization procedure is illustrated in scheme 1 (an alternative type of cyclisation will be described later with reference to scheme 2).



Scheme 1

If R_{11} is other than hydrogen, the compound of formula **I**, may be prepared by direct alkylation of the alcohol **Ia**. Compounds with Q=S can be prepared by treatment of the corresponding alcohol **Ia** with $R_{11}SH$, and a catalyst (usually a Lewis Acid such as Al_2O_3). For compounds where Q=NH, these can be prepared by reacting an amine, $R_{11}NH$, e.g. C_3H_7NH with the corresponding alcohol **Ia** normally with a catalyst, such as a Lewis Acid.

Exposure of the alcohol (**B**) to tetrapropylammonium perruthenate (TPAP)/N-methylmorpholine N-oxide (NMO) over A4 sieves results in oxidation accompanied by spontaneous B-ring closure to afford the desired product. The TPAP/NMO oxidation procedure is found to be particularly convenient for small scale reactions while the use of DMSO-based oxidation methods,

particularly Swern oxidation, proves superior for larger scale work (e.g. > 1 g).

The uncyclized alcohol (**B**) may be prepared by the reaction of the amino alcohol **C**, generally in solution, with the linking group L attached to a solid support (**D**). The linking group is preferably terminated with a chloroformate or acid chloride functionality. This reaction is generally carried out in the presence of a base such as pyridine (preferably 2 equivalents) at a low temperature (e.g. at 0°C).

The key amino alcohol **C** may be prepared by reduction of the corresponding nitro compound **E**, by choosing a method which will leave the rest of the molecule intact. For example, treatment of **E** with tin (II) chloride in a suitable solvent, e.g. refluxing methanol, generally affords, after the removal of the tin salts, the desired product **C** in high yield.

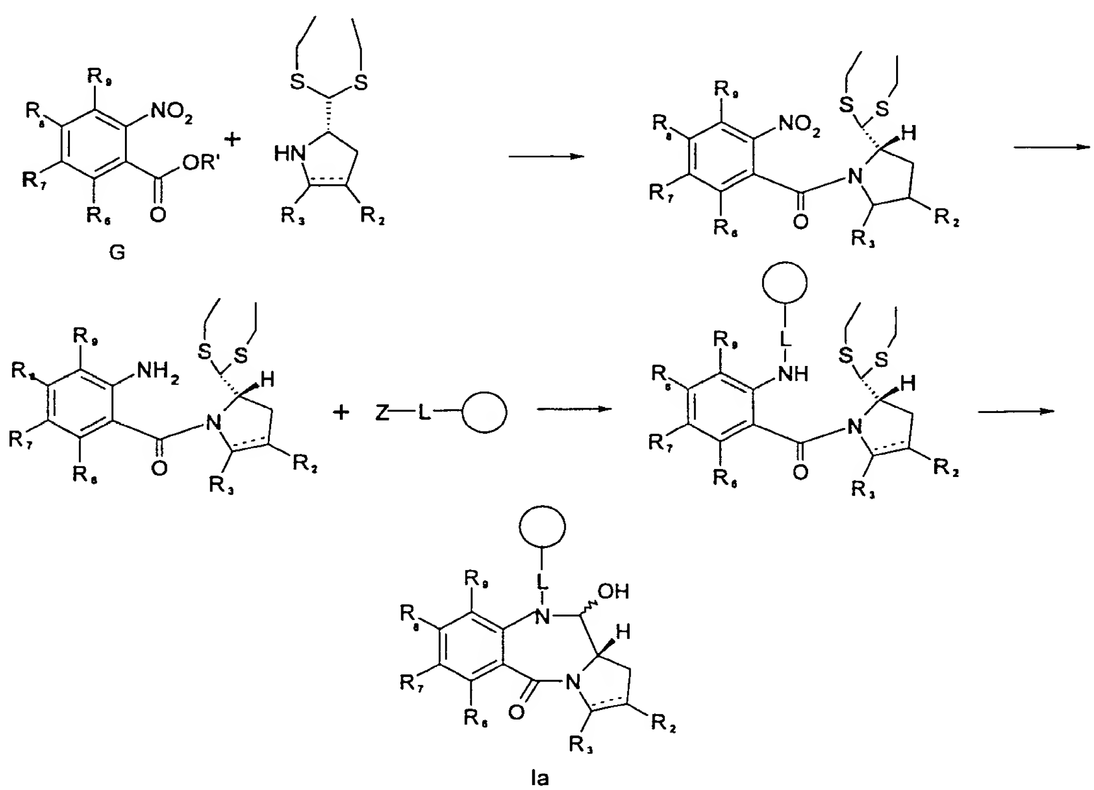
Exposure of **E** to hydrazine/Raney nickel avoids the production of tin salts and may result in a higher yield of **C**, although this method is less compatible with the range of possible C and A-ring substituents. For instance, if there is C-ring unsaturation (either in the ring itself, or in R_2 or R_3), this technique may be unsuitable.

The nitro compound of formula **E** may be prepared by coupling the appropriate *o*-nitrobenzoyl chloride to a compound of formula **F**, e.g. in the presence of K_2CO_3 at -25°C under a N_2

atmosphere. Compounds of formula **F** can be readily prepared, for example by olefination of the ketone derived from L-trans-hydroxy proline. The ketone intermediate can also be exploited by conversion to the enol triflate for use in palladium mediated coupling reactions.

The *o*-nitrobenzoyl chloride is synthesised from the o-nitrobenzoic acid (or alkyl ester, after hydrolysis) of formula **G**, which itself is prepared from the vanillic acid (or alkyl ester) derivative **H**. Many of these are commercially available and some are disclosed in Althuis, T.H. and Hess, H.J., J. Medicinal Chem, 20(1), 146-266.

Alternative Cyclisation (Scheme 2)



Scheme 2

In scheme 1, the final or penultimate step was an oxidative cyclisation. An alternative route, using thioacetal coupling, is shown in scheme 2. Mercury-mediated unmasking causes cyclisation to the desired compound (**Ia**).

The thioacetal compound may be prepared as shown in scheme 2: the thioacetal protected C-ring [prepared via a literature method: Langley, D.R. & Thurston, D.E., *J. Organic Chemistry*, **52**, 91-97 (1987)] is coupled to the o-nitrobenzoic acid (or alkyl ester) (**G**) using a literature procedure. The resulting nitro compound cannot be reduced by hydrogenation because of the thioacetal group, so the tin(II) chloride method is used to afford the amine. This is then N-protected, e.g., by reaction with a chloroformate or acid chloride, such as p-nitrobenzylchloroformate.

Acetal containing C-rings can be used as an alternative in this type of route with deprotection including other methods, including the use of Lewis Acid conditions(see example 3).

In the above synthesis schemes, the derivatisation of the A-ring is shown as being complete before the compounds are attached to the solid support. This is preferred if the substituents are groups such as alkoxy or nitro. On the other hand, substituent groups such as alkyl or alkenyl could be added to the A-ring after the coupling of the compound to the solid support. This may be achieved by R_6, R_7, R_8 or R_9 being easily replaceable groups, such as a halogen atom.

Embodiments of the present invention will now be described by way of example with reference to the accompanying drawings in which:

Figure 1 is a synthesis scheme for a compound according to the invention;

Figure 2 is a synthesis scheme for another compound according to the invention;

Figure 3 is a synthesis scheme for an intermediate in the synthesis of a compound according to the invention;

Figure 4 is a synthesis scheme for a further compound according to the invention;

Figure 5 is an HPLC time course for cleavage of the compound made by the scheme shown in figure 2;

Figure 6 is a graph which illustrates the results shown in figure 5;

Figure 7 is a graph illustrating the cytotoxicity of the compound made by the scheme shown in figure 2; and

Figure 8 is a synthesis scheme for a further compound according to the invention.

General Methods

Melting points (mp) were determined on a Gallenkamp P1384 digital melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Perkin-Elmer 297 spectrophotometer. ^{1}H- and ^{13}C- NMR spectra were recorded on a Jeol GSX 270 MHZ FT-NMR spectrometer operating at 20°C +/-1°C. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS). Spin multiplicities are described as: s (singlet), bs (broad singlet), d

(doublet), dd (doublet of doublets), t (triplet), q (quartet), p (pentuplet) or m (multiplet). Mass spectra (MS) were recorded using a Jeol JMS-DX 303 GC Mass Spectrometer (EI mode: 70eV, source 117-147°C). Accurate molecular masses (HRMS) were determined by peak matching using perfluorokerosene (PFK) as an internal mass marker, and FAB mass spectra were obtained from a glycerol/thioglycerol/trifluoroacetic acid (1:1:0.1) matrix with a source temperature of 180°C. Optical rotations at the Na-D line were obtained at ambient temperature using a Perkin-Elmer 141 Polarimeter. Analytical results were generally within +/-0.2% of the theoretical values. Flash chromatography was performed using Aldrich flash chromatography "Silica Gel-60" (E. Merck, 230-400 mesh). Thin-layer chromatography (TLC) was performed using GF_{254} silica gel (with fluorescent indicator) on glass plates. All solvents and reagents, unless otherwise stated, were supplied by the Aldrich Chemical Company Ltd. and were used as supplied without further purification. Anhydrous solvents were prepared by distillation under a dry nitrogen atmosphere in the presence of an appropriate drying agent, and were stored over 4Å molecular sieves or sodium wire. Petroleum ether refers to the fraction boiling at 60-80°C.

<u>Overall Synthetic Strategy for Examples 1 and 2</u>

The pyrrolobenzodiazepine products **8** and **13** were obtained in solution by exposure to light at 365 nm; light at this wavelength promotes the conversion of the photolabile linker

into a nitroso aldehyde, in the process liberating the PBD from the resin. In addition to this photolabile linker, other fluoride, mild acid, mild base or palladium(0)/nucleophile labile linkers may also be used in the construction of PBD libraries.

The bead bound PBDs **7** and **12** (figures 1 and 2 respectively) were prepared by oxidation of the primary-alcohol-bearing resins **6** and **11** with SO_3.Pyridine complex in DMSO. Other oxidizing systems such as TPAP/NMO, the Dess Martin reagent, and oxalyl chloride/DMSO (Swern oxidation) are also effective. The primary alcohol resins **6** and **11** were obtained from the coupling of the bead bound anthranilic acids **4** and **10** to pyrrolidine methanol (**5**). Alternatively, coupling (2*S*, 4*R*)-2-t-butyldimethylsilyloxymethyl-4-hydroxy proline to the bead-bound anthranilic acid offers the opportunity of elaborating the PBD C-ring at the pro-C2-position on bead *via*, for example, olefination. Finally, the bead bound anthranilic acids **4** and **10** were obtained by coupling the commercially available anthranilic acids (over 40 anthranilic acids are commercially available) to the o-nitrobenzylchloroformate resin **2** which was in turn obtained from the commercially available resin **1** by treatment with triphosgene in the presence of dry pyridine.

Example 1: Synthesis of the Resin-Bound C7-Iodo-PBD Carbinolamine (7) (Figure 1)

Acid Chloride Resin (**2**)

Hydroxymethyl-photolinker NovaSyn TG resin **1** (0.2 g, 0.24 mmol/g loading) was placed in a vessel, fitted with a sinter. Dichloromethane CH_2Cl_2 (3 mL) was added and the vessel shaken for 30 min. The suspension was then cooled to 0°C before adding triphosgene (0.15 g, 0.5 mmol) in CH_2Cl_2 and pyridine (40 µL, 0.5 mmol), and the vessel allowed to shake at room temperature for 16 h. The chloroformate resin **2** was collected by filtration and rinsed with CH_2Cl_2 (2 x 5 mL) and MeOH (2 x 5 mL), and dried *in vacuo*. IR (reflectance, cm^{-1}): 1700 (C=O).

Attaching Iodinated A-Ring to form resin (**4**)

Dichloromethane CH_2Cl_2 (5 mL) was added to resin **2** (0.048 mmol) and the vessel was allowed to shake for 30 min. The suspension was then cooled to 0°C and a solution of iodoanthranilic acid **3** (0.13 g, 0.48 mmol) and pyridine (40 µl) in NMP (2 mL) was added, and the vessel was allowed to shake at room temperature for 16 h. Resin **4** was then collected by filtration, rinsed with CH_2Cl_2 (2 x 5 mL), NMP (2 x 5 mL) and MeOH (2 x 5 mL), and dried *in vacuo*. HPLC analysis after release of iodoanthranilic acid by irradiation indicated that 58% of available sites had been carbonylated. IR (reflectance, cm^{-1}): 1750-1650 (CONH).

Attaching Pyrrolo C-Ring to from resin (6)

Dimethyl formamide DMF (5 mL) was added to resin **4** (0.036 mmol) and the vessel allowed to shake for 30 min. Pyrrolidine methanol **5** (40 µl, 0.36 mmol), TBTU (0.12 g, 0.36 mmol) in DMF (1 mL) and DIPEA (65 µl, 0.36 mmol) were added, and the vessel allowed to shake at room temperature for 16 h. Resin **6** was collected by filtration, rinsed with CH_2Cl_2 (2 x 5 mL) and MeOH (2 x 5 mL), and dried *in vacuo*. IR (reflectance, cm^{-1}): 1650-1600 (C=O).

B-Ring Cyclisation to form Bead-Bound Carbinolamine (7)

Dichloromethane CH_2Cl_2 (0.5 mL) was added to resin **6** (0.024 mmol) and the vessel allowed to shake for 30 min. The suspension was then cooled to -10°C, and triethylamine (10 µl, 0.072 mmol) and sulphur trioxide.pyridine complex (0.012 g, 0.072 mmol) in DMSO (0.25 mL) added. Shaking was continued for 1 h at 10°C, and the resin (**7**) was then collected by filtration, rinsed with CH_2Cl_2 (2 x 5 mL) and MeOH (2 x 5 mL), and dried *in vacuo*.

The resulting compound **7** may be cleaved from the solid support by UV light of a wavelength of 365 nm to form a compound of formula **8**.

Further synthesis steps

The compound of formula **7** may serve as a starting point for the synthesis of a wide variety of other compounds. The iodine at the C8 position can be reacted with a boronic acid

with $Pd(PPh_3)_4$ as a catalyst in modified Suzuki reaction. An alternative synthesis route is to stanylate the C8 position by reacting the compound of formula **8** with Me_6Sn_2, with $Pd(PPh_3)_4$ as a catalyst. The stanylated compound is capable of coupling with acrylates (i.e. the Heck reaction), iodo- and bromo-arenes (i.e. the Suzuki reaction) and haloalkenes (i.e. the Stille reaction).

Example 2: Synthesis of Resin-bound 7,8-Dimethoxy PBD (12) (Figure 2)

Attaching Dimethoxy A-Ring to form Resin (**10**)

Dichloromethane CH_2Cl_2 (2 mL) was added to the choloroformate resin **2** (0.05 mmol) (prepared as in example 1) and the vessel allowed to shake for 30 min. The suspension was cooled to 0°C, a solution of 4,5-dimethoxyanthranilic acid (**9**) (0.05 g, 0.25 mmol) and pyridine (20 µL) in NMP (2 mL) added, and the vessel allowed to shake at room temperature for 16 h. The resin **10** was collected by filtration, and then rinsed with CH_2Cl_2 (2 x 5 mL), NMP (2 x 5 mL) and MeOH (2 x 5 mL). The entire procedure was repeated twice and the resin was then dried *in vacuo*. IR (reflectance, cm^{-1}): 1750-1650 (CONH).

Attaching Pyrrolo C-Ring to form Resin (**11**)

Dimethyl formamide DMF (5 mL) was added to resin **10** (0.05 mmol) and the vessel allowed to shake for 30 min. Pyrrolidine methanol (**5**) (0.025 g, 0.25 mmol), TBTU (0.08 g, 0.25 mmol) in DMF (1 mL) and DIPEA (45 µL, 0.25 mmol) were added, and the vessel allowed to shake at room temperature for 16 h. The

resin **11** was collected by filtration, and rinsed with DMF (2 x 5 mL), NMP (2 x 5 mL) and CH_2Cl_2 (2 x 5 mL). The entire procedure was repeated twice, and the resin then dried *in vacuo*. IR (reflectance, cm^{-1}): 1700-1600 (C=O).

B-Ring Cyclisation to form Bead-Bound Carbinolamine (**12**)

Dichloromethane CH_2Cl_2 (1 mL) was added to resin **11** (0.05 mmol) and the vessel allowed to shake for 30 min. The suspension was cooled to -10°C, and triethylamine (20 µL, 0.15 mmol) and sulphur trioxide.pyridine complex (0.024 g, 0.15 mmol) in DMSO (0.5 mL) were added. The suspension was then allowed to warm to room temperature, and the vessel was left to shake for 2 h. The resin **12** was collected by filtration and rinsed with CH_2Cl_2 (2 x 5 mL) and MeOH (2 x 5 mL). The entire procedure was repeated twice and the resin then dried *in vacuo*.

Example 3: Alternative Synthesis of Resin-bound 7,8-Dimethoxy PBD (21) (Figures 3 & 4)

Overall Synthetic Strategy

The on-bead oxidation step employed in the previous approaches can be avoided by coupling an anthranilic acid loaded resin to the dimethyl acetal (**16**) derived from proline (figure 4). In this approach, unmasking of the dimethyl acetal protected aldehyde leads to spontaneous B-ring closure. Thus, exposure of the acetal **20** to a palladium catalyst ($Pd(CH_3CN)_2Cl_2$) leads to the formation of the cyclized compound **21**. The acetal **20** was derived from the anthranilic acid resin **19** and the acetal **16**, which were coupled together under standard conditions.

The acetal **16** was obtained from the Cbz protected compound **15** (figure 3) by hydrogenation; **15** was in turn prepared by acetalisation of the aldehyde **14**. Swern oxidation of the primary alcohol **13** afforded the aldehyde **14**, the primary alcohol was prepared by a lithium tetrahydroborate reduction of the commercially available Cbz protected proline ester ester **12**.

(2*S*-N-(benzoxvcarbonyl)-2-hydroxymethylproline (**13**)

Lithium tetrahydroborate (2.6 g, 0.12 mol) was added portionwise to a solution of N-Carbobenzyloxy-L-proline methyl ester **12** (21 g, 0.08 mol) in THF (500 mL) at 0°C. The reaction mixture was allowed to stir at room temperature for 48 h. The solution was then cooled to 0°C and ice water (150 mL) was added to quench excess lithium tetrahydroborate. The resulting suspension was adjusted to pH 4.0 with aqueous HCl (1.0 N) and extracted with Et_2O (250 mL). The organic phase was separated and washed with H_2O (3 x 100 mL), brine (2 x 100 mL), dried ($MgSO_4$) and concentrated to give alcohol **13** as a pale yellow oil (18.6 g, 99%). ^1H NMR (270 MH_z, $CDCl_3$) δ 2.1-1.77 (m, 4H); 3.76-3.35 (m, 4H); 4.1-3.77 (m, 1H); 5.14 (2 x s, 2H); 7.38-7.28 (m, 5H). CIMS 236 (M^+).

(2*S*)-N-benzoxycarbonyl)pyrrolidine-2-carboxaldehyde (**14**)

A solution of triethylamine (32 mL, 0.23 mol) and SO_3.pyridine complex (37 g, 0.23 mol) in DMSO (210 mL) a solution of alcohol **13** (18 g, 0.077 mol) in CH_2Cl_2 (250 mL) at -10°C, under a nitrogen atmosphere. The reaction mixture was allowed to

warn to room temperature and stirred for 30 min and then poured into ice water (200 mL) and extracted with Et_2O. The organic phase was washed with aqueous HCl (1.0 N, 3 x 150 mL), H_2O (3 x 150 mL), brine (2 x 150 mL), dried ($MgSO_4$) and concentrated to give a yellow oil. The crude material was purified by flash column chromatography (EtOAc) to give aldehyde **14** as a colourless oil (12.6 g, 71%). ^1H NMR (270 MH_z, $CDCl_3$) δ 2.16-1.8 (m, 4H); 3.66-3.5 (m, 2H); 4.22-4.17 (m, 1H); 5.22-5.13 (m, 2H); 7.37-7.3 (m, 5H); 9,59 (2 x s, 1H). CIMS 234 (M^+ + 1).

(2*S*)-N-(benzoxycarbonyl)pyrrolidine-2-carboxaldehyde dimethyl acetal (**15**)

Thionyl chloride (5.5 mL) was added to a solution of aldehyde **14** (11 g, 0.047 mol) and trimethyl orthoformate (36 mL, 0.33 mol) in MeOH (55 mL) at 0°C. The reaction mixture was heated at 60°C for 2 h. The solution was allowed to cool to room temperature, and treated with excess solid Na_2CO_3 and diluted with Et_2O (60 mL). The suspension was filtered to remove insoluble inorganics and resultant filtrate was concentrated *in vacuo* and the redissolved in EtOAc. The organic solution was washed with saturated aqueous $NaHCO_3$ (3 x 50 mL), brine (2 x 50 mL), dried ($MgSO_4$) and concentrated to give the acetal **15** as a yellow liquid (12.5 g, 95%). ^1H NMR (270 MH_z, $CDCl_3$) δ 2.16-17 (m, 4H); 3.64-3.33 (m, 4.02-3.91 (br. m, 1H); 4.4 and 4.6 (2 x br. s, 1H); 4.4 and 4.6 (2 x br. s, 1H); 5.17-5.1 (m, 2H); 7.47-7.28 (m, 5H).

Pyrrolidine-2-carboxaldehyde dimethyl acetal (**16**)

A solution of acetal **15** (5.8 g, 0.02 mol) in EtOH (50 mL) was allowed to stir for 16 h at room temperature over Raney nickel (0.2 g), in order to remove the trace amounts of sulphur impurities prior to hydrogenation. Excess nickel was removed by filtration through Celite.

10% palladium on carbon (580 mg) was added to the alcoholic solution which was subjected to hydrogenation under pressure (c. 50 psi). After 16 h, the reaction mixture was filtered through Celite and the pad washed with EtOAc, the combined organic solutions were concentrated to give the secondary amine **16** as a pale green liquid (2.9 g, 100%). ^{1}H NMR (270 MH_{Z}, $CDCl_3$) δ 1.93-1.59 (m, 4H); 3.1-2.92 (m, 2H); 3.4-3.3 (d, J = 6.9, 1H); 3.41 (2 x s, 6H); 3.53 (br. s, 1H); 4.2 (d, J = 6.8, 1H).

Synthesis of Resin-bound Methyl Ester **21** (Figure 4)

A suspension of hydroxyethyl-photolinker NovaSyn TG resin **17** (0.114 g, 0.24 mmol/g loading) in CH_2Cl_2 (1 mL) in a vessel fitted with a sinter was shaken for 30 min. The suspension was cooled to 0°C, before addition of a solution of triphosgene (0.04 g, 0.14 mmol) and pyridine (11 mL, 0.14 mmol) in CH_2Cl_2 (0.5 mL). The vessel was allowed to shake at room temperature for 16 h. The resin **18** was filtered and rinsed with CH_2Cl_2 (2 x 2 mL) NMP (2 x 2 mL) and CH_2Cl_2 (2 x 2 mL). This procedure was repeated twice and the resin was then dried *in vacuo*.

A suspension of resin **18** (0.027 mmol) in CH_2Cl_2 (1 mL) was allowed to shake for 30 mins. The suspension was cooled to 0°C and a solution of 4,5-dimethoxy-anthranilic acid **8** (0.03 g, 0.14 mmol) and pyridine (10 mL) in NMP (0.5 mL) was added. The vessel was allowed to shake at room temperature for 16 h.

Resin **19** was filtered and rinsed with CH_2Cl_2 (2 x 2 mL), NMP (2 x 2 mL) and MeOH (2 x 2 mL). The procedure was repeated twice and then the resin was dried *in vacuo*.

A suspension of resin **19** (0.027 mmol) in DMF (1 mL) was allowed to shake for 30 min. To this suspension was added acetal **16** (20 mg, 0.14 mmol), TBTU (43 mg, 0.14 mmol) and DIPEA (25 mL, 0.144 mmol) in DMF (0.5 mL). The vessel was allowed to shake at room temperature for 2 h after which time the resin **20** was filtered and rinsed with DMF (2 x 1 mL), CH_2Cl_2 (2 x 1 mL) and MeOH (2 x 1 mL). The procedure was repeated twice and then the resin was dried *in vacuo*.

A suspension of resin **20** (0.027 mmol) in acetone (0.5 mL) was allowed to shake for 30 min. To this suspension was added $PdCl_2(CH_3CN)_2$ (7 mg, 0.027 mmol) in acetone (0.4 mL) and the vessel was allowed to shake at room temperature for 2 h. The resulting resin **21** was filtered and rinsed with acetone (2 x 1 mL), CH_2Cl_2 (2 x 1 mL) and MeOH (2 x 1 mL). The procedure was repeated twice and then the resin was dried *in vacuo*.

Example 4: Cleavage of PBDs from beads

HPLC Method

Assays of the PBDs synthesised in example 2 were carried out on a reversed-phase 25cm x 4.6mm (inside diameter) C4 (Nucleosil™; 5μm bead size) column protected with a Delta-Pak™ C4, 300Å Guard pre-column. Elution was carried out using a mobile phase consisting of $MeOH/H_2O$ (1:1) at a flow rate of 1mL/min. A Waters 490E multiwavelength detector was used. Peak identification was accomplished by reference to an authentic sample of compound **13** synthesized "off-bead".

Conditions

INJECTION VOLUME:	20μL
FLOW RATE:	1mL/min
MOBILE PHASE:	50% METHANOL/50% WATER
STATIONARY PHASE:	C4, 5μm (REVERSED PHASE)
COLUMN:	WATERS 300Å
DETECTOR:	254nm
RUN TIME:	20mins

Cleavage of the PBD from beads following UVA irradiation was monitored by HPLC. The resin-bound compound **12** (JMB 98) at a concentration of 1mM in DMF was UVA irradiated. At appropriate time intervals, samples were centrifuged to pellet the beads and the amount of free PBD released into the supernatant determined by HPLC. After photolysis of resin **12**, carbinolamine **13** was the only species produced as determined by reference to an authentic sample of compound **13** synthesised "off bead". Typical HPLC traces of authentic **13** and of the PBD cleaved from resin **12** with increasing irradiation times are shown in Figure 5, and the percentage cleavage with time shown in Figure 6. Cleavage occured linearly with time, and complete cleavage was achieved

by 2 hours under the conditions used. HPLC studies indicated that 77% of the sites on the beads had reacted.

In Vitro Cytotoxicity Assay

MTT Assay Method

The ability of agents to inhibit the growth of chronic human histiocytic leukaemia U937 cells or human chronic myeloid leukaemia K562 cells in culture was measured using the MTT assay (Mosmann, 1983). This assay is based on the ability of viable cells to reduce a yellow soluble tetrazolium salt, 3-(4,5-dimethylthiazolyl)-2,5-diphenyltetrazolium bromide (MTT; Sigma Chemical Co.), to an insoluble purple formazan precipitate. Cells at a density of 5 x 104 cells/mL were continuously incubated with the test compounds at a final concentration of 0.3μM. Aliquots of each of the compounds of the 27-memeber library were either left without UVA (365 nm) exposure or were exposed to UVA (365 nm) for 2 hours prior to their addition to the cell suspension. Following drug treatment, the cells were transferred to 96-well microtitre plates, 10^4 cells per well, 8 wells per sample. The plates were incubated at 37°C in a humidified atmosphere containing 5% CO_2. Following incubation of the plates for 4 days (to allow control cells to increase in number 10-fold), 20μL of a 5mg/mL solution of MTT in phosphate-buffered saline was added to each well and the plates further incubated for 5 hours. The plates were then centrifuged for 5 min at 300g, and the bulk of the medium removed from the cell pellet, leaving 10-20μL per well. DMSO (200μL) was added to each well, and the samples agitated to ensure complete mixing. The optical density was then read

at a wavelength of 550nm using a Titertek Multiscan ELISA plate reader and the dose-response curve constructed. The IC_{50} value was read as the dose required to reduce the final optical density to 50% of the control value.

Results

The cytotoxicity of the PBD released from resin **12** following irradiation was determined using the MTT assay. The survival curve resulting from the compound released from **12** (JMB 98) following 2 and 5 hours irradiation was consistent with that of authentic **13** (AG 105); see figure 7. The released PBD therefore has full biological activity.

Example 5: Synthesis of a resin-bound 8-aminopropyl PBD (30) (see Figure 8)

Overall Synthetic Strategy

The o-nitrobenzylchloroformate resin (2) can also immobilize more complicated amines other than simple anthranilic acids, greatly facilitating the preparation of molecules such as the PBD C8-amino scaffold **30**. As in the previous strategy, the Fmoc protected scaffold **29** was prepared by oxidizing the primary alcohol resin **28**. This resin was obtained by loading the o-nitrobenzylchloroformate resin (**2**) with the amino alcohol (**27**). The amino alcohol was prepared by a Tin (II) chloride mediated reduction of the nitro alcohol **26**; use of hydrogenation conditions to reduce the nitro group were avoided due to the presence of the Fmoc group in **26**. The nitro alcohol in turn was furnished in this case by coupling

pyrrolidine methanol (**5**) to the o-nitrobenzoic acid **25**, although other functionalised prolines could also be employed in the coupling reaction The Fmoc o-nitrobenzoic acid was obtained via Fmoc protection of the amino acid **24** produced by hydrolysis of the ester **23**.. Other nitrogen protecting groups may be substituted for Fmoc as long as the cleavage conditions involved are compatible with the presence of an o-nitrobenzyl carbamate linker (eg. Boc, Alloc, Teoc etc). Finally, the amino ester was prepared by nitration of **22** which was obtained by a Mitsunobu etherification of commercially available methyl vanillate.

Boc Amino Ester (**22**)

A solution diethylazidodicarboxylate (3.38 g, 19.4 mmol) in THF (50 mL) was added dropwise to a solution of methylvanillate (3.53 g, 19.4 mmol), N-Boc-propanolamine (3.4 g, 19.4 mmol) and triphenylphosphine (5.09 g, 19.4 mmol) in THF (50 mL) at 0°C. The reaction mixture was allowed to warm to room temperature and stir overnight. Excess solvent was removed by rotary evaporation under reduced pressure and the residue triturated with toluene. Precipitated triphenylphosphine oxide was removed by vacuum filtration and the filtrate concentrated *in vacuo*. The residue was subjected to flash column chromatography (silica gel, petroleum ether 40 - 60/ethyl acetate, 80/20) and removal of excess eluent afforded the pure product **22** (4.8 g, 73 % yield.). ^1H NMR (270 MHz, $CDCl_3$) δ 7.65 (dd, J = 8.43, 2.02 Hz, 1H), 7.54 (d, J = 2.02 Hz, 1H), 6.86 (d, J = 8.43 Hz, 1H), 5.55 (bs, 1H), 4.15

(t, J = 5.87 Hz, 2H), 3.93 (s, 3H), 3.90 (s, 3H), 3.41-3.35 (m, 2H), 2.09-2.00 (m, 2H) and 1.46 (s, 9H). ^{13}C NMR (68.7 MHz, $CDCl_3$) δ 166.9, 156.1, 152.1, 148.8, 123.5, 122.8, 112.0, 111.2, 79.0, 68.2, 55.9, 52.0, 38.9, 29.2 and 28.5.

Amino Nitro Ester (**23**)

The Boc-protected amine **22** (10 g) was added portionwise to cold nitric acid (30 mL, 70%, ice bath), the reaction mixture was allowed warm to room temperature and stir overnight. The reaction mixture was poured onto crushed ice (100 g) and the resulting aqueous solution reduced to half its original volume by rotary evaporation under reduced pressure. The resulting precipitate was collected by vacuum filtration and recrystallised from absolute ethanol to afford the product as a yellow crystalline solid **23** (8.9 g, 87%). ^1H NMR (270 MHz, $CDCl_3$) δ 7.47 (s, 1H), 7.08 (s, 1H), 4.24 (t, J = 5.86 Hz, 2H), 3.96, (s, 3H), 3.89 (s, 3H), 3.24 (t, J = 6.78, 2H) and 2.32-2.23 (m, 2H).

Amino Nitro Acid (**24**)

A solution of potassium hydroxide (0.5 g, 8.7 mmol) and the nitrobenzoic acid **23** (1 g, 2.9 mmol) in aqueous methanol (H_2O, 10 mL; methanol, 20 mL) was allowed to stir at room temperature for 1 h and then heated at reflux until TLC (AcOEt, MeOH, TEA, 1:10:100) revealed the complete consumption of starting material. Excess methanol was removed by rotary

evaporation and the residual solution diluted with water and neutralised with 1N HCl. The neutralised aqueous solution was used directly, without further purification, in the next synthetic step.

Fmoc Nitro Acid (**25**)

Fluorenylmethyl chloroformate (0.78 g, 3 mmol) was added portionwise to the aqueous solution from the previous reaction which had been diluted with THF (50 mL) and aqueous sodium carbonate (2.15 g, 50 mL water). The reaction mixture was then allowed to stir overnight. Excess organic solvent was removed by rotary evaporation under reduced pressure from the reaction mixture, the residual aqueous solution was then washed with ethyl acetate (3 x 20 mL) (to remove excess Fmoc-Cl). The aqueous phase was acidified with conc. HCl and extracted with ethyl acetate (2 x 50 mL). The organic phase was dried over magnesium sulphate, filtered and evaporated *in vacuo* to afford the product **25** (1 g, 70% yield). ^{1}H NMR (270 MHz, $CDCl_3$) δ(Rotamers) 8.21 (bs, 2H), 7.73 (d, J = 7.14 Hz, 2H), 7.59 (d, J = 7 .33, 2H) 7.40 - 7.13 (m, 5H), 6.47 and 5.70 (2 x bs, 1H), 4.54-3.88 (m, 5H), 3.77 (s, 3H), 3.44-3.42 (m, 2H) and 2.04-1.90 (m, 2H). ^{13}C NMR (68.7 MHz, $CDCl_3$) δ 168.7, 156.9, 152.1, 149.8, 143.7, 141.9, 141.3, 127.7, 127.0, 124.9, 120.6, 120.0, 111.1, 107.8, 68.5, 66.4, 56.4, 47.3, 39.1 and 28.4.

Fmoc Nitro Alcohol (**26**)

A catalytic amount of DMF (2 drops) was added to a solution of the acid **25** (1.16 g, 2.36 mmol) and oxalyl chloride (0.33 g,

2.6 mmol) in dry dichloromethane (20 mL) and the reaction mixture was allowed to stir overnight. The resulting acid chloride solution was cooled to 0°C and treated dropwise with a solution of pyrrolidinemethanol (0.26 g, 2.57 mmol) and triethylamine (0.52 g, 5.14 mmol) in dry dichloromethane (15 mL). Thin layer chromatography, performed shortly after the end of the addition of amine, revealed that reaction had gone to completion. The reaction mixture was washed with HCl (1N, 1 x 50 mL) and water (2 x 20 mL) and dried over magnesium sulphate. Removal of excess solvent afforded the crude product which was subjected to flash column chromatography (silica gel, gradient elution,1% methanol in chloroform to 2% methanol in chloroform) to afford the required amide **26** (1.1 g, 81%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.75 (d, J = 7.33 Hz, 2H), 7.67 (s, 1H), 7.60 (d, J = 6.96 Hz, 2H), 7.41-7.26 (m, 4H), 6.78 (s, 1H), 5.66 (bs, 1H), 4.48-4.39 (m, 3H), 4.23-4.13 (m, 3H), 3.91-3.79 (m, 5H), 3.45-3.42 (m, 2H), 3.18-3.13 (m, 2H) and 2.08-1.70 (m, 6H). ^{13}C NMR (68.7 MHz, $CDCl_3$) δ 168.5, 156.5, 154.7, 148.2, 143.9, 141.3, 137.0, 128.0, 127.7, 127.0, 124.9, 120, 108.9, 108.0, 68.4, 66.2, 66.0, 61.5, 56.6, 53.5, 47.3, 39.0, 28.9, 28.4 and 24.4.

Fmoc Amino Alcohol **(27)**

A solution of the nitroamide **26** (3 g, 5.22 mmol) and $SnCl_2$ $2H_2O$ (6.15 g, 27.15 mmol) in methanol (60 mL) was heated at reflux for 2 h. The reaction mixture was concentrated to 1/3 of its original volume and carefully treated with saturated aqueous sodium bicarbonate solution (vigorous effervescence!) until pH8 was obtained. The mixture was allowed to stir vigorously

with ethyl acetate (100 mL) overnight and then filtered through celite to remove precipitated tin salts. The aqueous phase was extracted with ethyl acetate (50 mL) and the combined organic phase was dried over magnesium sulphate. Removal of excess solvent afforded the desired amine as a dark yellow oil **27** (1.93 g, 68%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.75 (d, J = 7.51, 2H), 7.61 (d, J = 7.33 Hz, 2H), 7.40-7.26 (m, 4H), 6.72 (s, 1H), 6.25 (s, 1H), 5.95 (bs, 1H), 4.43-4.04 (m, 6H), 3.67-3.42 (m, 9H) and 2.11-1.7 (m, 6H). ^{13}C NMR (68.7 MHz, $CDCl_3$) δ 171.7, 156.6, 150.8, 144.0, 141.3, 140.6, 127.6, 127.0, 125.0, 119.9, 112.0, 102.2, 68.0, 66.6, 66.4, 61.0, 56.6, 51.0, 47.3, 39.5, 29.1, 28.5 and 24.9.

Resin-bound amino-alcohol (**28**)

The *o*-nitrobenzylchloroformate resin (0.048 mmol) was allowed to swell for 10 minutes in dry dichloromethane (5 mL). A solution of the amino alcohol (0.13 mg, 0.24 mmol) and pyridine (0.02 g) in dry dichloromethane (1 mL) was added to the resin suspension under a nitrogen atmosphere at 0°C. The reaction mixture was then allowed to shake overnight at room temperature. Excess reagent was removed by suction and the resin washed with dichloromethane (2 x 5 mL) and methanol (2 x 5 mL) and then dried *in vacuo* overnight. The procedure was repeated three times to ensure complete reaction.

Resin-bound Fmoc-aminopropyl PBD (**29**)

The carbamate resin (0.048 mmol) prepared in the previous reaction was allowed to swell in dry dichloromethane (1 mL) for 10 minutes. The suspension was cooled to -10°C and treated

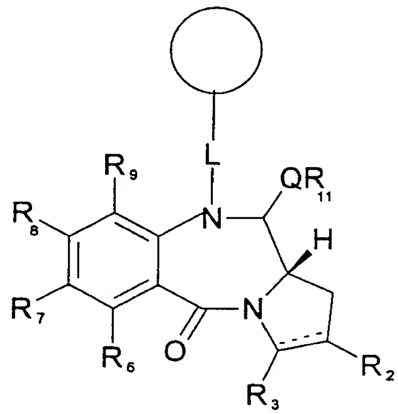
successively with triethylamine (20 μL, 0.144 mmol) and pyridine sulphur trioxide complex (0.023 g, 0.144 mmol) in dimethyl sulphoxide (0.5 mL) at -10°C and the suspension was allowed to shake at -10°C for two hours. Excess reagent was removed by suction and the resin washed with methanol (2 x 5 mL) and dichloromethane (2 x 5 mL) and then dried *in vacuo* overnight. The procedure was repeated three times to ensure complete reaction.

Resin-bound Aminopropyl PBD (30)

The resin-bound 8-aminopropyl PBD (**30**) is prepared from the Fmoc-protected form (**29**) by standard deprotection conditions. This compound **30** can be used to form compounds according to the second aspect of the present invention, by reaction of appropriate combinatorial units from those described above with this compound.

CLAIMS

1. A compound of formula (I):



(I)

wherein:

R_2 and R_3 are independently selected from: H, R, OH, OR, =O, =CH-R, $=CH_2$, CH_2-CO_2R, CH_2-CO_2H, CH_2-SO_2R, $O-SO_2R$, CO_2R, COR and CN, and there is optionally a double bond between C2 and C3;

R_6, R_7, R_8 and R_9 are independently selected from H, R, OH, OR, halo, nitro, amino, Me_3Sn;

R_{11} is either H or R;

Q is S, O or NH;

L is a linking group, or a single bond;

O is a solid support;

where R is a lower alkyl group having 1 to 10 carbon atoms, or an aralkyl group of up to 12 carbon atoms, whereof the alkyl group optionally contains one or more carbon-carbon double or triple bonds, which may form part of a conjugated system, or an aryl group of up to 12 carbon atoms; and is optionally substituted by one or more halo, hydroxy, amino, or nitro groups, and optionally contains one or more hetero atoms,

which may form part of, or be, a functional group.

2. A compound according to claim 1, wherein one or more of R_2, R_3, R_6, R_7 and R_8 are independently X-Y-A-, where X is selected from -COOH, NHZ, SH, or OH, where Z is either H or an amine protecting group, Y is a divalent alkylene or aryl group such that HY = R, and A is O, S, NH, or a single bond.

3. A compound according to claim 2, wherein it is either R_2 and/or R_8 that is X-Y-A-.

4. A compound according to claim 1, wherein one or more of R_2, R_3, R_6, R_7 and R_8 are independently:

$$H-(T)_n-X'-Y-A-$$

where:

X' is CO, NH, S or O,;

T is a combinatorial unit;

and n is a positive integer.

5. A compound according to claim 4, wherein it is R_2 and/or R_8 that are independently:

$$H-(T)_n-X'-Y-A-.$$

6. A compound according to claim 4 or claim 5, wherein X' is either CO or NH.

7. A compound according to any one of claims 4 to 6, wherein n is from 1 to 16.

8. A compound according to claim 7, wherein n is from 3 to 14.

9. A compound according to any one of the preceding claims, wherein R, and HY if Y is present, are independently selected from lower alkyl group having 1 to 10 carbon atoms, or an alkaryl group of up to 12 carbon atoms, or an aryl group of up to 12 carbon atoms, optionally substituted by one or more halo, hydroxy, amino, or nitro groups.

10. A compound according to claim 9, wherein R, and HY, if Y is present, are independently selected from lower alkyl group having 1 to 10 carbon atoms optionally substituted by one or more halo, hydroxy, amino, or nitro groups.

11. A compound according to claim 10, wherein R, and HY, if Y is present, are unsubstituted straight or branched chain alkyl groups, having 1 to 10 carbon atoms.

12. A compound according to any one of the preceding claims, wherein Q is O.

13. A compound according to any one of the preceding claims, wherein R_{11} is H.

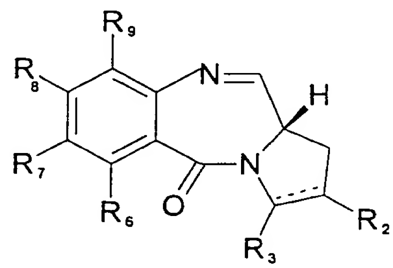
14. A compound according to any one of the preceding claims, wherein R_6 and R_9 are H.

15. A compound according to any one of the preceding claims,

wherein R_7 is an alkoxy group.

16. A compound according to any one of the preceding claims, wherein R_2 and R_3 are H.

17. A compound of formula **II**:



(II)

wherein R_2, R_3, R_6, R_7, R_8 and R_9 are as defined in any one of claims 1 to 16.

18. A compound of formula **II** as defined in claim 17 for use in a method of therapy.

19. A pharmaceutical composition, comprising a compound of formula **II** as defined in claim 17, and a pharmaceutically acceptable carrier or diluent.

20. The use of a compound of formula **II** as defined in claim 17 in the preparation of a medicament for the treatment of a gene-based disease.

21. The use of a compound of formula **II** as defined in claim 17 in the preparation of a medicament for the treatment of bacterial, parasitic or viral infections.

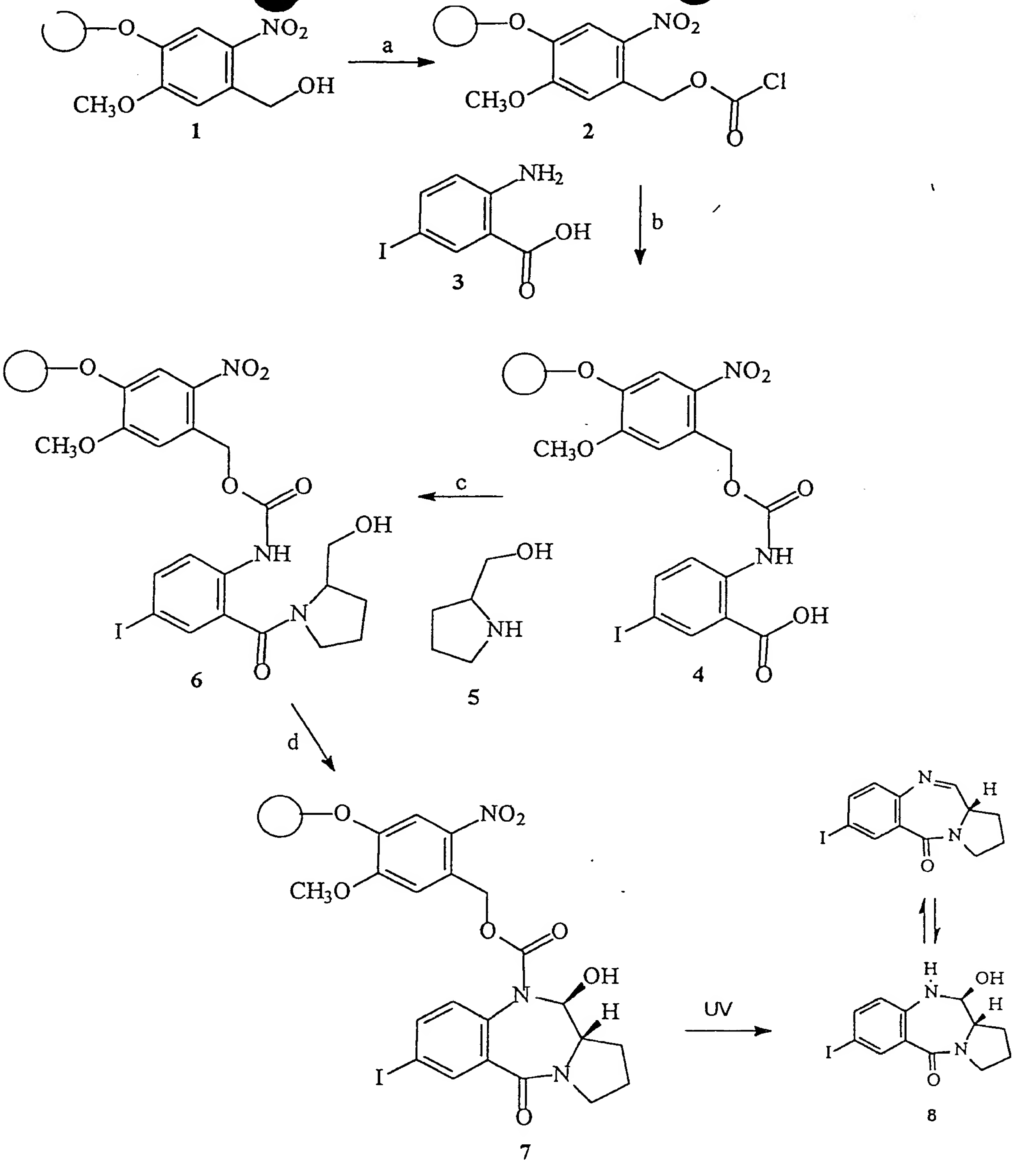
22. A collection of compounds all of which are represented by formula I as defined in any one of claims 1 to 16.

23. A collection of compounds all of which are represented by formula II as defined in claim 17, wherein R_2, R_3, R_6, R_7 and R_8 are as defined in any one of claims 4 to 8, or in any one of claims 9 to 11, 14, 15, or 16 as appendant, directly or indirectly, on any one of claims 4 to 8.

24. A method of preparing a collection of compounds as defined in either claims 22 or 23.

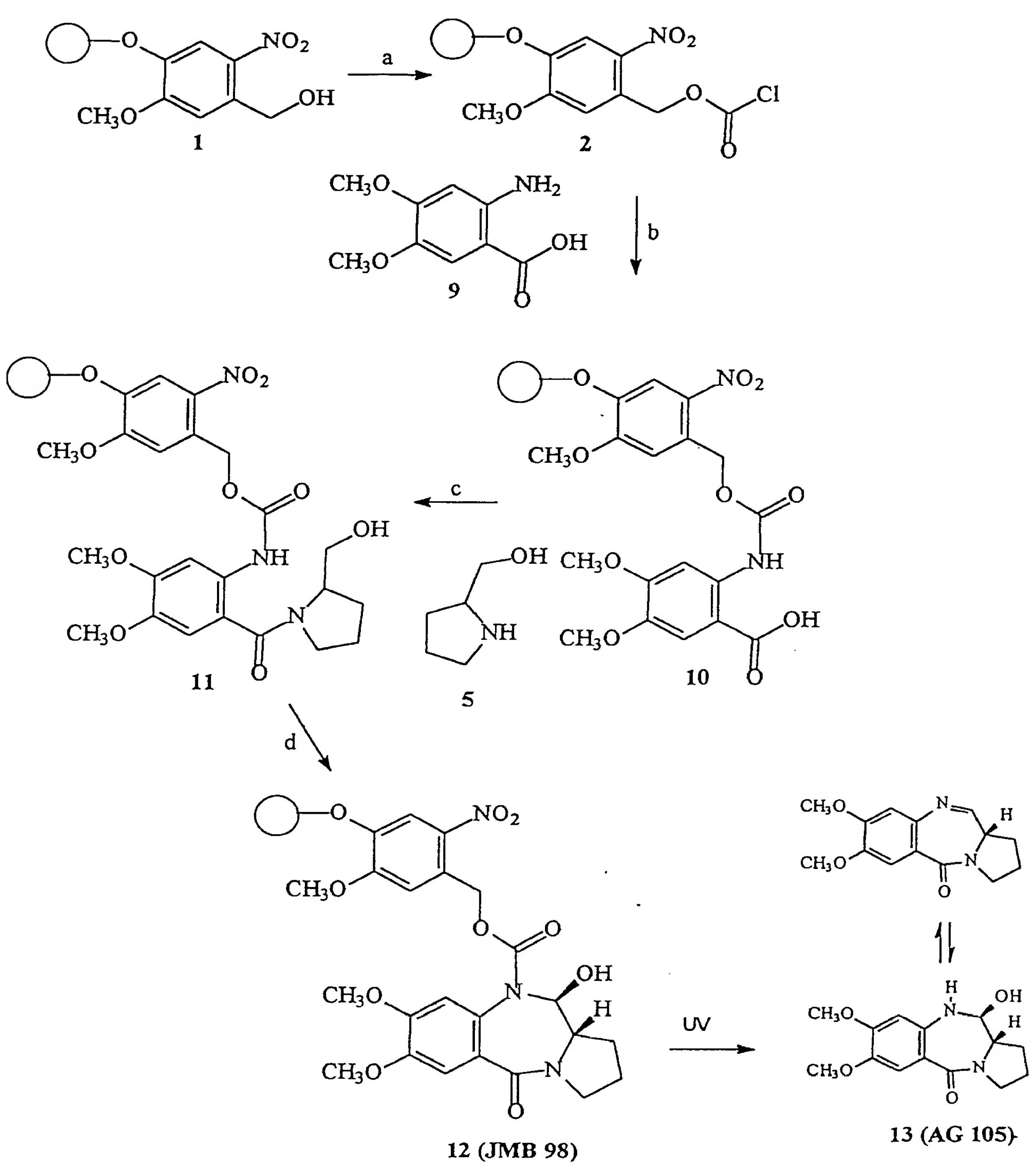
25. A method of screening a collection of compounds of formula II as defined in claim 23 to discover biologically active compounds.

26. The use of a compound of formula II as defined in claim 17 in a method of target validation.



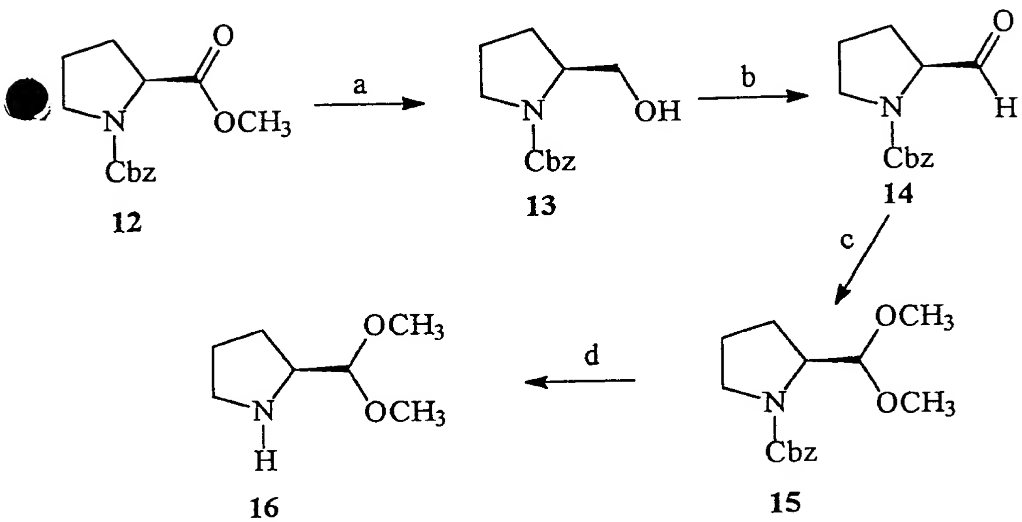
Reagents. a: Triphosgene, pyridine, CH_2Cl_2; b: pyridine, CH_2Cl_2; c: TBTU, DIPEA, DMF; d: SO_3.pyridine, TEA, CH_2Cl_2, DMSO.

Fig. 1



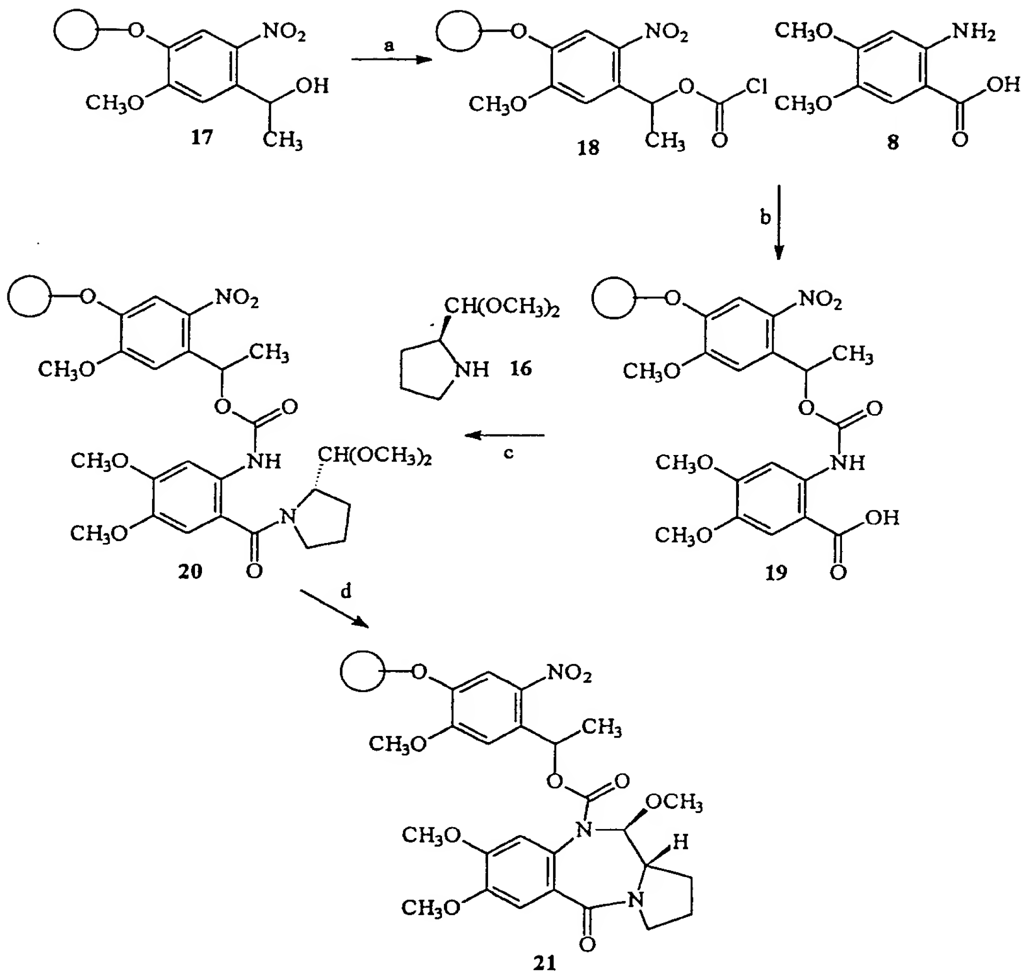
Reagents. a: Triphosgene, pyridine, CH_2Cl_2; b: pyridine, CH_2Cl_2; c: TBTU, DIPEA, DMF; d: SO_3.pyridine, TEA, CH_2Cl_2, DMSO.

Fig. 2



a: $LiBH_4$, THF; b: SO_3.pyridine, TEA, CH_2Cl_2, DMSO; c: MeOH, $SOCl_2$, $CH(OCH_3)_3$; d: (i) Raney Nickel, EtOH, (ii) H_2, Pd-C, EtOH.

Fig. 3



NO2
OH
CH3O
17
CH3
a
NO2
O
Cl
CH3O
18
CH3
O
CH3O
NH2
OH
CH3O
8
O
b
NO2
CH3
CH3O
O
O
CH3O
NH
CH3O
OH
19
O
CH(OCH3)2
NH
16
c
NO2
CH3
CH3O
O
O
CH3O
NH
CH(OCH3)2
CH3O
N
20
O
d
NO2
CH3
CH3O
O
O
OCH3
N
H
CH3O
CH3O
N
O
21

3

Fig. 4

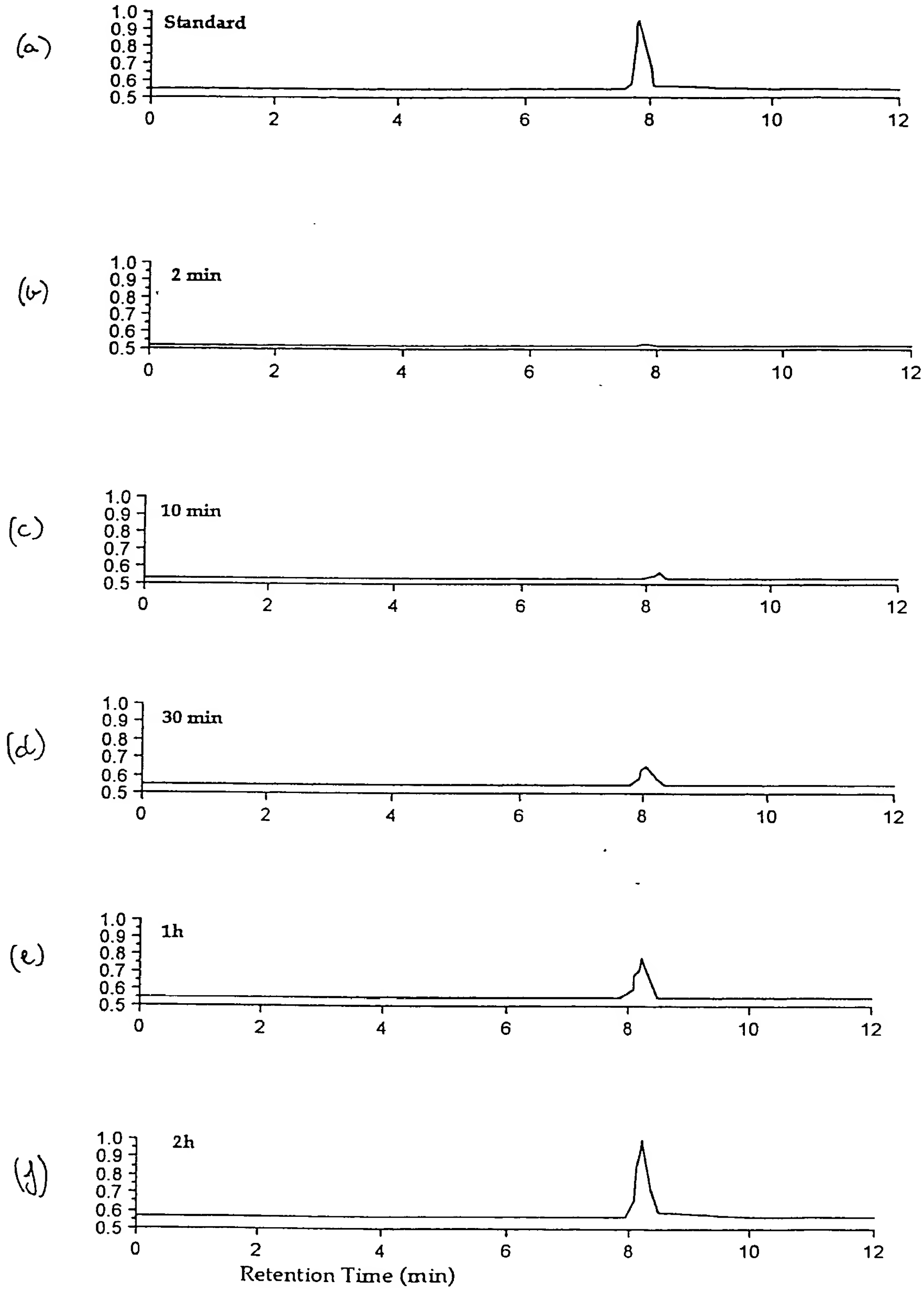
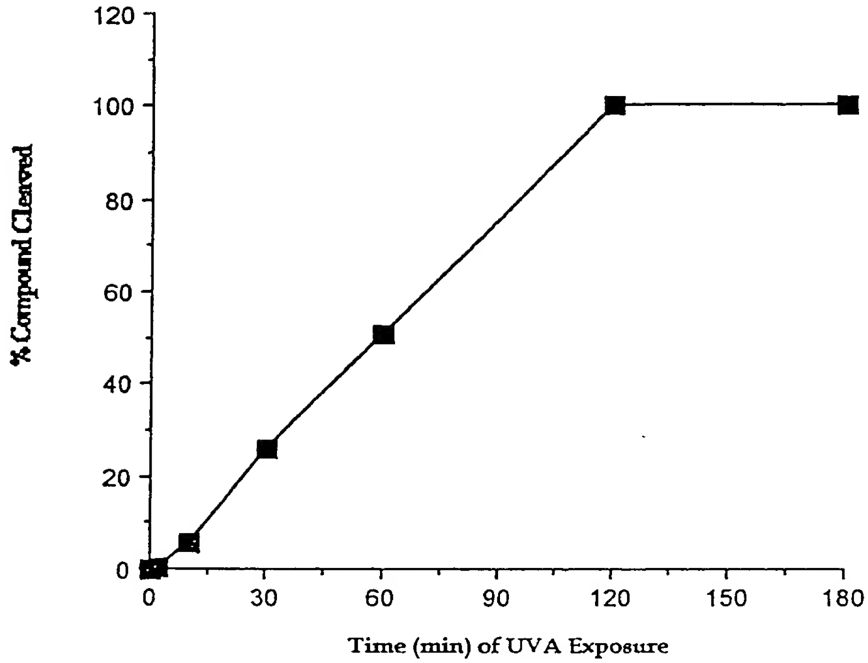
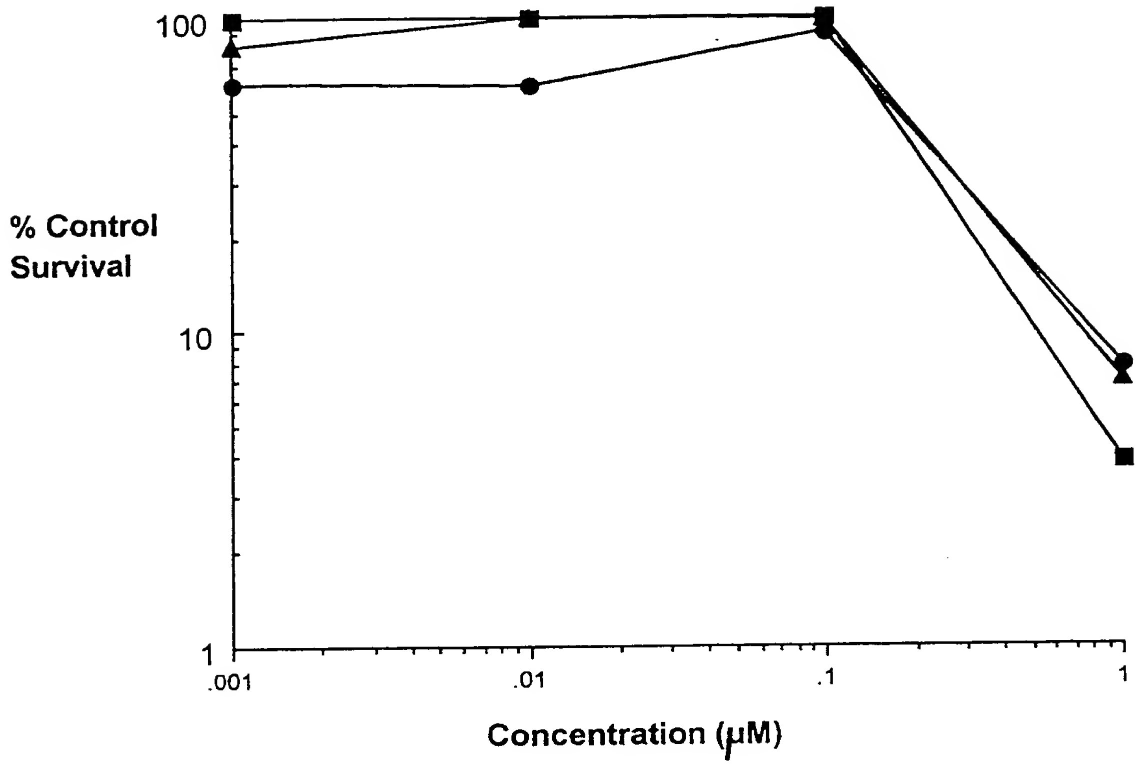

(a)
Standard
(b)
2 min
(c)
10 min
(d)
30 min
(e)
1h
(f)
2h
1.0
0.9
0.8
0.7
0.6
0.5
0
2
4
6
8
10
12
Retention Time (min)

Fig. 5



120
100
80
60
40
20
0
% Compound Cleaved
0
30
60
90
120
150
180
Time (min) of UVA Exposure

Fig. 6



In vitro cytotoxicity assay for AG 105 (squares); compound **12** + UVA 2h (circles) and compound **12** + UVA 5h (triangles).

Fig. 7

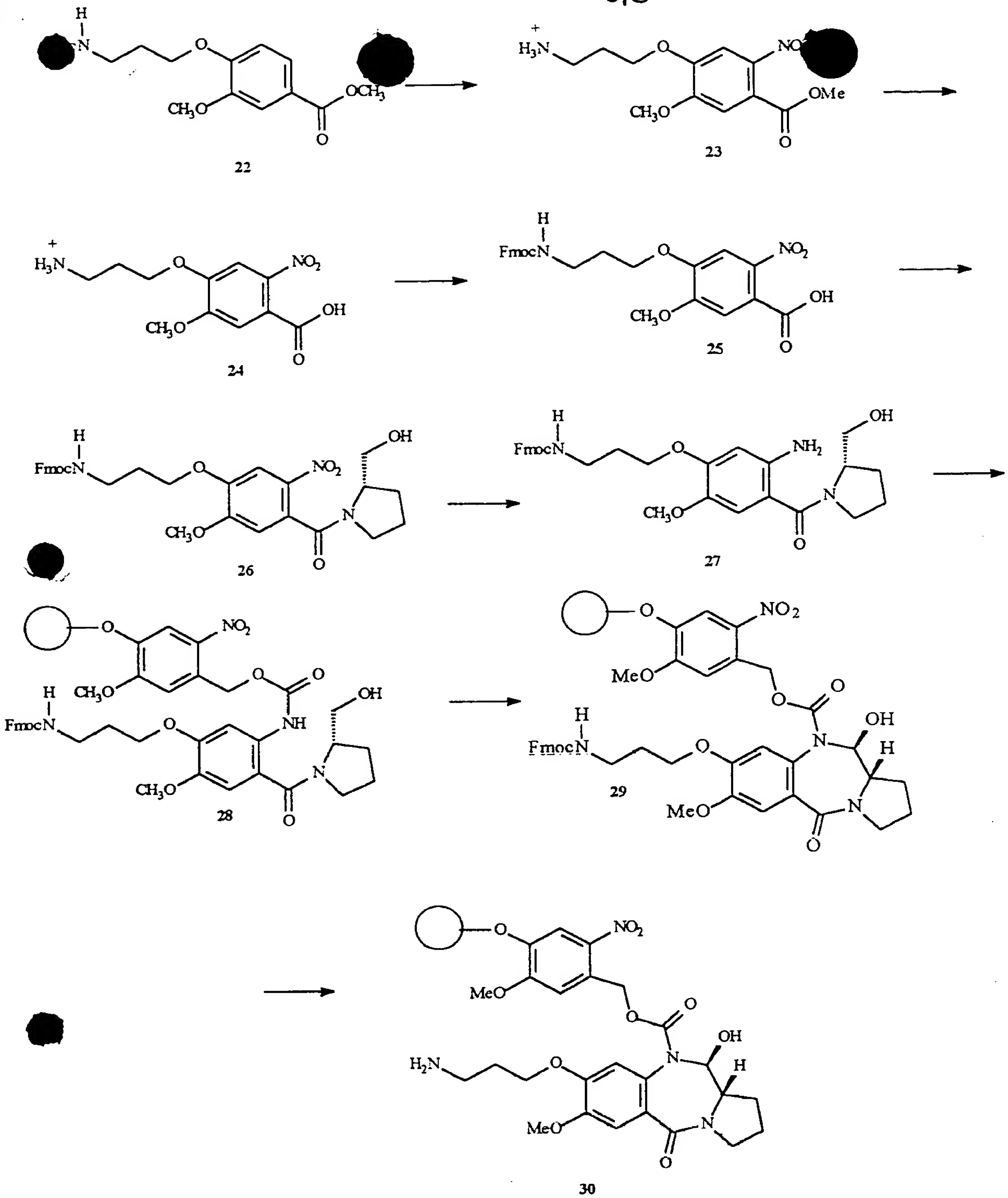

22
23
24
25
26
27
28
29
30
NO2
OCH3
OMe
CH3O
MeO
FmocN
H3N
H2N
NH2
NH
OH
H
O
N

Fig. 8.

PCT No : GB99 / 02839

FORM 23/77 : 27/8/99

AGENT : Mewburn Ellis